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Characterization of Electrode Fouling and Surface Regeneration for a Platinum Electrode on an Electrophoresis Microchip

Drew P. Manica,[†] Yutaro Mitsumori,[‡] and Andrew G. Ewing*,[†]

Department of Chemistry, 152 Davey Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16801, and Department of Biomolecular Engineering, Tohoku University, Aramaki 07, Aoba, Sendai 980-8579, Japan

A method of electrochemically cleaning noble metal electrodes is presented and characterized for electrophoresis microchips with electrochemical detection. First, the loss of sensitivity due to electrode fouling by serotonin is characterized as a function of injection number and analyte concentration. Signal attenuation is observed to be greater at high concentrations (100 μM) and negligible at very low concentrations (∼1 μM). Next, an electrochemical treatment procedure is optimized to yield sensitive and reproducible amperometric detection of the highly adsorptive compounds, serotonin and histamine. Thus, the performance of the electrode is reproducibly regenerated following as much as a ∼99% reduction in surface activity. Utilizing the optimized three-level waveform, derived from that used for pulsed amperometric detection, detection limits as low as 78 nM and 17 μM have been obtained for serotonin and histamine, respectively. In the case of serotonin, this represents the lowest detection limit for a neurotransmitter by microchip electrophoresis with amperometric detection and the first report of amperometric detection of histamine detection at an unmodified platinum electrode. Repeated use of the electrode and application of electrochemical treatment did not appear to measurably affect the noise, longevity, metal adhesion, or physical appearance of the electrode.

The development of micrototal analysis systems (μTAS, or lab-on-a-chip) has led to many benefits, such as portability, disposability, high efficiency, decreased analysis time, and the means to analyze ultrasmall samples with little dilution (see reviews).[1−5] An ideal μTAS involves the consolidation of all analytical stages—sample preparation, separation, and detection—onto a single platform.[6] Electrochemical detection is an attractive method to utilize for microchip analyses owing to its ease of fabrication onto planar substrates, sensitivity, and selectivity for important analytes, such as neurotransmitters. Since the introduction of electrochemical detection for planar electrophoresis devices,[7] the use of microchip electrophoresis with electrochemical detection (microchip CEEC) has rapidly expanded to include amperometry,[8] voltammetry,[9,10] and conductimetry.[11] Likewise, the materials used for electrodes on chips have also expanded to include the following: platinum, gold, copper, palladium, carbon fiber, carbon paste, screen-printed carbon, gold-modified screen-printed carbon, palladium-modified screen-printed carbon, mercury-modified gold, platinum wire, and copper-coated platinum (see reviews).[12−15] Each electrode surface interacts uniquely with different analytes, possibly resulting in unexpected reactions or attenuation of signal over time and use. Therefore, the solution to achieving reproducible results is to consider possible analyte−surface interactions when the electrode material is selected and to incorporate some form of electrode-cleaning regimen if necessary.

Platinum is one of the most widely used materials for amperometric detection on microchips, owing partly to its robustness, versatility, and classification as a noble metal. While Pt may be a noble metal and more inert than many surfaces, it is certainly capable of undergoing oxidation and adsorbing gases and hydrocarbons. From a practical standpoint, this means it may not be viewed simply as a sink of electrons.[16,17] The oxidation of Pt to PtO, the adsorption of hydrogen gas, and the adsorption of organics are a few of the reaction pathways that compete with the desired faradaic reaction at the electrode.

* Corresponding author. E-mail: age@psu.edu. Phone: (814) 863-4653. Fax: (814) 863-8081.

[†] The Pennsylvania State University.

[‡] Tohoku University.

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Because these adverse reactions cannot be avoided completely, it is essential that any treatment of the electrode be duplicated so that the competing reactions are normalized across measurements in order to maintain precision. For Pt electrodes in bulk solution, this has been accomplished by routine chemical[16] or electrochemical[18] treatment of the electrode. In the case of chemical treatment, the surface is first strongly oxidized using an oxidizing agent such as dichromate or permanganate, followed by dissolution of the oxide layer using reducing agents such as ferrous sulfate or As-(II).[16] The exact treatment conditions vary with the intended application, but the common theme of oxide formation and oxide dissolution is conserved. The general principle of electrochemical cleaning is analogous to chemical cleaning. The utility of electrical potential waveforms to clean noble metal electrodes has long been known;[16] however, it was the work of Johnson and LaCourse[18,19] that led to the development and characterization of electrochemical potential pulses to clean platinum and gold electrodes. In this procedure, the working electrode is first stepped up to a large positive potential. During this phase, an oxide layer (PtO or AuO) is formed on the surface, and any adsorbed organic material is simultaneously stripped off of the surface. The removal of the adsorbate results in an increase in current, which has been used for detection of carbohydrates, amino acids, alcohols, and glycols by pulsed amperometric detection (PAD).[18] Following formation of the oxide and removal of adsorbate, the oxide layer is cathodically dissolved by applying a reducing potential to the electrode. This serves to regenerate the clean, oxide-free noble metal surface, after which the potential is returned to the operating voltage of the specific electrochemical couple.

Historically, various forms of electrochemical treatment have been used to clean electrodes for amperometric detection on a microchip. Woolley et al.[20] adopted a regimen of shorting the platinum working electrode and silver wire pseudoreference electrode (at 0 mV) for 70 s prior to detection of neurotransmitters at +800 mV. This was reported to maintain signal reproducibility; however, electrode treatment was not a focus of the study, and no evidence was given for the efficacy of the technique. It may be assumed from the theory discussed above that this cleaning paradigm may have had limited ability to desorb any organic material but may have been effective in removing surface oxides. In a separate study, Gawron et al.[21] applied a bipolar square wave of ±1.8 V (30 Hz for 30 s) to carbon fiber electrodes that intersected a PDMS channel. In this way, catechol peak heights were restored from a 19% reduction after 41 injections back to 100% of the initial peak height. In the same laboratory, Martin et al.[22] applied the same bipolar square wave to thin-film gold electrodes, reportedly restoring catechol response to 98% of the original signal. Bipolar square waves of this amplitude historically have been applied to carbon electrodes to remove adsorbed species, create an oxygen-rich layer on the electrode, and significantly roughen the carbon surface.[23] While this waveform may be appropriate for carbon electrodes, and may have removed electrogenerated products at a gold electrode, it may be too harsh for thin-film noble metal electrodes. It has been reported that the use of such a rigorous treatment destroys the thin-film gold electrode, typically limiting its lifetime to ~150 separations over 8 h.[22]

While a small number of investigators have incorporated electrochemical treatment paradigms to regenerate the electrode surface in microchip CEEC,[20−22] a complete study has not been devoted to the topic of electrode fouling and surface regeneration for microchip analyses. Given the recent growth of microchip CEEC, it is apparent that the ability to overcome surface-related challenges is profoundly important. This is especially true in light of the recent trend toward applying microchip CE systems to cellular analysis,[24−33] since biological compounds and cellular matter are notorious for their tendency to strongly adsorb to metal surfaces. As microchip CEEC continues to advance into the arena of cellular analysis, it is important to develop strategies for cleaning the electrode surface to enable analytical measurements of cellular systems.

One important cell system used to model exocytosis is the mast cell. Mast cells play an important role in immune response by storing and releasing histamine (Him), serotonin (5-HT), and immunoglobulins.[34,35] Histamine and serotonin are biological amines that adsorb strongly to metal surfaces, complicating their quantitative electrochemical measurement. There are no reports of direct amperometric detection of Him at an unmodified Pt electrode, and there is only one report of amperometric detection of 5-HT on a microchip.[21] Both these analytes form byproducts that adsorb and therefore foul the electrode surface when oxidized. Amperometric detection of Him typically has been accomplished at carbon electrodes,[36] boron-doped diamond electrodes,[37] or enzyme-modified metal electrodes[35,38] to lessen electrode fouling. Gawron et al.[21] demonstrated amperometric detection of 5-HT on a microchip using a carbon fiber electrode intersecting a PDMS channel. In that study, the fouling of the electrode from electrogenerated products of 5-HT was not addressed. Without a suitable method to reproducibly clean the electrode surface, the applications of microchip CEEC remain limited.

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This paper describes the characterization of electrode fouling and surface regeneration of an unmodified Pt metal electrode to accomplish sensitive and precise measurement of 5-HT and Him on a microchip. Electrode fouling is characterized as a function of injection number and analyte concentration. The efficacy of the electrochemical cleaning procedure is optimized and applied to the detection of 5-HT and Him. The use of a PAD waveform to treat a Pt electrode on a chip for surface regeneration is novel and results in the lowest detection limit for a neurotransmitter using microchip CEEC to date.

EXPERIMENTAL SECTION

Electrophoresis Apparatus and Procedures. Glass electrophoretic microchips with integrated platinum electrodes were fabricated in-house at the EMPRL Nanofabrication Facility (Pennsylvania State University, University Park, PA) as described previously.[8] Microchips employed a three-way cross-tee injector, with the separation channel length measuring 2.66 cm and the buffer and sample arms measuring 0.35 and 0.30 cm, respectively. The channels were 9.0 μm deep and 32.0 μm wide at full width. The working electrode was manually aligned approximately 40−50 μm from the separation channel with the aid of a microscope before thermal bonding. Sample introduction was performed by applying 1.0 kV between the sample and detection reservoirs, floating the buffer reservoir. The separation was performed by applying 1.0 kV between the buffer and detection reservoirs, floating the sample reservoir.

End-column amperometric detection was performed using a two-electrode cell as previously reported.[8] Briefly, a 250-μm-diameter silver wire (Goodfellow, Huntingdon, England), oxidized in 1 M KCl solution, was used as a pseudoreference electrode and placed ~50 μm from the integrated thin-film working electrode as observed under a microscope (200-Å Ti adhesion layer, 2000-Å Pt electrode layer). The working electrode was maintained at +800 or +950 mV (vs Ag/AgCl) for detection of 5-HT or Him, respectively, using a battery-operated floating potentiostat made in-house. The signal was amplified (model 427, Keithley Instruments, Cleveland, OH) and collected at 50 Hz using an in-house program written in Labview (National Instruments, Austin, TX).

Reagents and Solutions. Reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. 2-(*N*-Morpholino)ethanesulfonic acid (MES; 25 mM, pH adjusted to 6.6) was prepared for use as run buffer. Stock solutions of 5-HT (10 mM) and Him (100 mM) were prepared in perchloric acid (100 mM) to prevent oxidation in solution and diluted to the desired concentration with MES buffer (25 mM) immediately prior to use. All solutions were filtered (0.2 μm) before use.

Electrode Treatment. Electrochemical treatment of the platinum working electrode was accomplished by applying a programmed waveform to the working electrode versus the reference electrode. The electronic circuitry was configured such that the reference and working electrodes were connected through a toggle switch to either the detection electronics or the waveform generator (model 33120A, Hewlett-Packard, Loveland, CO). In this manner, the pulse waveform was applied only between runs and while rinsing all reservoirs with run buffer. During separation and detection of samples, the electrochemical cell was operated at constant potential for amperometric detection.

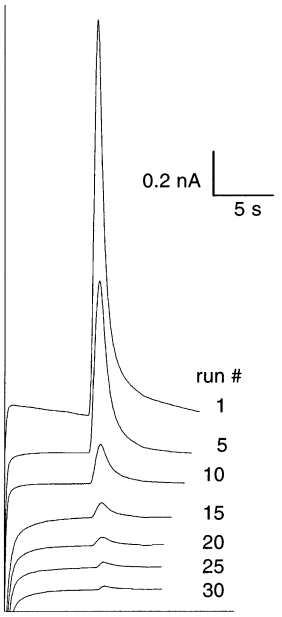

Figure 1. Selected electropherograms obtained over 30 serial injections of 100 μM 5-HT without cleaning between injections. Due to electrode fouling, the peak height is attenuated 98.9 ± 0.1% (±SD, $n = 3$) after 30 injections.

RESULTS AND DISCUSSION

To investigate treatment at Pt electrodes, a multistep potential waveform, similar to that used for PAD,[18,19] was applied to the working electrode. This treatment serves to strip off any adsorbed species and regenerate a reproducible, oxide-free electroactive surface, as described by Johnson and LaCourse[18] The three-level waveform used in this study includes a brief stabilization time (10% of period) at the detection potential ($E_1 = +800$ mV). Long stabilization times normally used for PAD[18,19] are avoided because there is no measurement phase during pulse application. Next, the electrode is oxidatively cleaned by applying a positive step ($E_2 = +1.2$ V) to the electrode for 10% of the period. This serves to form a surface oxide, PtO, and simultaneously desorbs organic compounds that may have adsorbed during use or disuse of the electrode. The resulting PtO surface is relatively inert and must be dissolved off by stepping down to a cathodic potential ($E_3 = 0$ V), thereby reactivating the native, oxide-free, noble metal surface.

Electrode Fouling with Serotonin and Histamine. For many analytes, electrochemical oxidation at noble metal electrodes is facilitated by the partially unsaturated surface d-orbitals that stabilize the intermediate transition products. By definition, these functional groups are strongly adsorptive, leading to an accumulation of oxidation products on the electrode surface. For many molecules that are difficult to detect with traditional amperometry, adsorption is a prerequisite to detection.[18] However, the adsorbed species may quickly occupy a substantial portion of the electroactive area, thereby "fouling" the electrode. Figure 1 illustrates the loss of signal over 30 sequential injections of 100 μM 5-HT with no cleaning waveform applied between injections. The electrode was prepared immediately prior to use by applying the waveform described above at 30 Hz for 30 s while running buffer through all reservoirs of the chip. The peak height was observed to reduce by 57.9 ± 2.1% (± SD, $n = 3$) after 5 injections, 89.9 ± 2.1% (±SD, $n = 3$) after 10 injections, and 98.9 ± 0.1% (±SD, $n =$

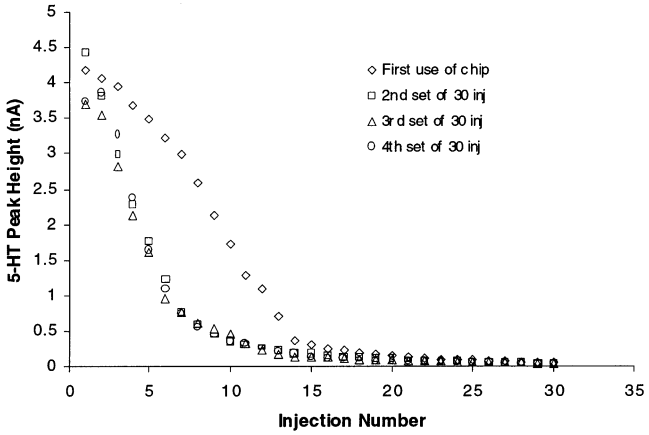

Figure 2. Peak height vs injection number for 30 injections of 100 μM 5-HT ($n = 4$). The symbols indicate the first (◇), second (□), third (Δ), and fourth (○) sets of 30 injections. The cleaning potential waveform was applied (30 Hz, 30 s) only between sets of injections.

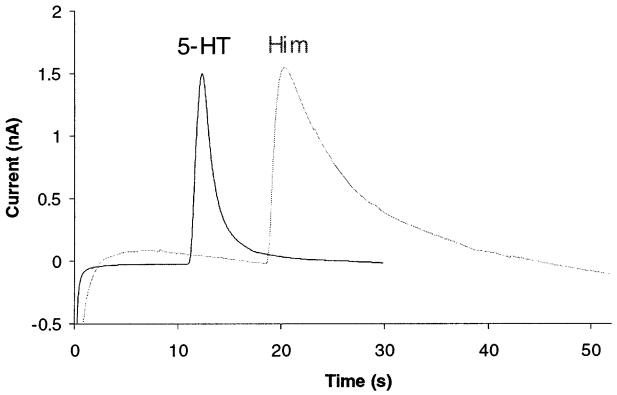

Figure 3. Comparative electropherograms of 100 μM serotonin and 100 μM histamine. The tailing observed for histamine may indicate greater adsorption of histamine on the electrode surface.

3) after 30 injections. Furthermore, the fouling of the electrode by serotonin oxidation products was observed to reduce the sensitivity to other species, regardless of their propensity to foul the electrode. Catechol (100 μM) peak heights were reduced by 95.8 ± 0.5% (±SD, $n = 3$) after 30 injections when injected together with 100 μM 5-HT, compared to a 23.9 ± 2.0% (±SD, $n = 3$) reduction using catechol alone (data not shown). Without cleaning the electrode, such a rapid and dramatic reduction in overall sensitivity would prohibit the application of the device to any systems with one or more highly adsorptive species.

After fouling, the electrode can be reproducibly regenerated by applying the stripping waveform. Figure 2 compares the loss of response observed from repeated detection of 100 μM 5-HT at a brand new electrode versus the same electrode after electrochemical treatment prior to each series of 30 injections. The response during the very first use of the chip differs from that observed following fouling and cleaning. According to Adams,[16] platinum electrodes almost always yield reproducible results provided the pretreatment of the electrode is duplicated with each use. Hence, it is not surprising that the surface of the electrode (and thus, its response) is different during its first use (after fabrication, chemical cleaning, storage, and high-temperature bonding) than after pretreatment. Prior to electrochemical treatment, it is likely that the surface had become coated with a variety of adsorbed species including organics, hydrogen, and surface oxides, and this surface could not be reproduced experimentally, except by fabricating new electrodes. It is important to note, however, that the behavior of the electrode was reproducible following electrochemical treatment, as indicated by the overlap of the three consecutive sets of 30 injections. Therefore, the electrochemical treatment described appears to be an appropriate means of erasing so-called "history effects".

Serotonin and histamine exhibit noticeably different electrode responses. Figure 3 illustrates comparative electropherograms of 100 μM 5-HT and 100 μM Him, demonstrating significant tailing of Him, which may be a qualitative indicator of analyte adsorption for amperometric detection. Aside from adsorption effects, numerous instrumental factors have been shown to contribute to undesirable peak attributes in microchip CE systems, such as injection methodology[39] and the alignment of the working and reference electrodes with respect to the separation channel.[8,20,40,41] It has been observed that serotonin and, to a greater extent, histamine exhibit significantly more tailing than catechol for a given chip, electrode alignment, and injection routine. Hence, it appears that much of the observed tailing is due to analyte adsorption. Additionally, concentrations of Him as high as 5 mM often result in no peak or asymmetric peaks that range from slightly negative to slightly positive when brand new chips are used without prior electrochemical cleaning. Despite this effect, applying electrochemical treatment restores reproducible positive peaks.

Concentration Dependence of Electrode Fouling. The attenuation of signal from fouling decreases with decreasing analyte concentration to a point below which fouling is negligible. This concept is demonstrated in Figure 4, in which 10 serial injections of 1 μM 5-HT result in relatively constant peak heights (17.8 ± 0.1 pA). The electrode was electrochemically treated (30 Hz, 30 s) prior to the first run only. The initial rise at the beginning of each injection is a typical electrochemical response when the run voltage is initiated and appears exaggerated here due the scale of the peak. Reproducible measurements can be accomplished with minimal surface treatment at very low concentrations.

Figure 5A illustrates the concentration dependence of electrode fouling over several concentrations of 5-HT (1, 10, and 100 μM). The peak height (±SD, $n = 3$) was monitored for the first 10 injections at each concentration, typically the time of greatest change in response. Again, the electrode was electrochemically treated before each set of 10 injections but not between each injection. For the concentrations tested, the greatest amount of attenuation of signal is observed for 100 μM 5-HT, and the least is observed for 1 μM. This can be clearly illustrated by plotting the slope between each injection versus injection number for each concentration as shown in Figure 5B. The significance of Figure 5B is that the first injections at high concentrations have greatly negative slopes, while almost all injections at the lowest concentration are within one standard deviation of zero slope.

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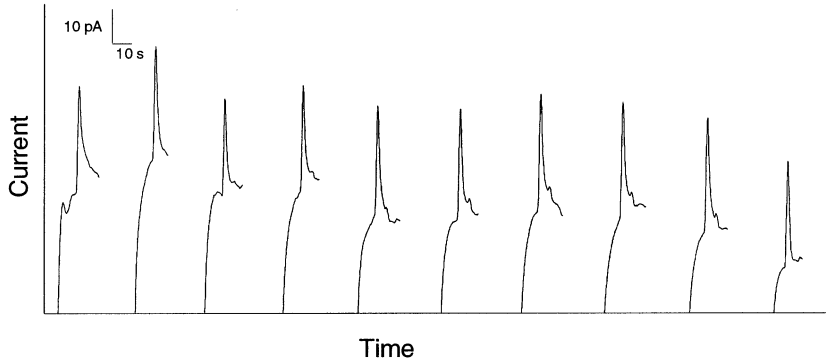

Figure 4. Ten serial injections of 1 μM 5-HT with no cleaning potential waveform applied between injections, demonstrating constant peak heights (17.8 $\pm$ 0.1 pA) for low concentrations of 5-HT.

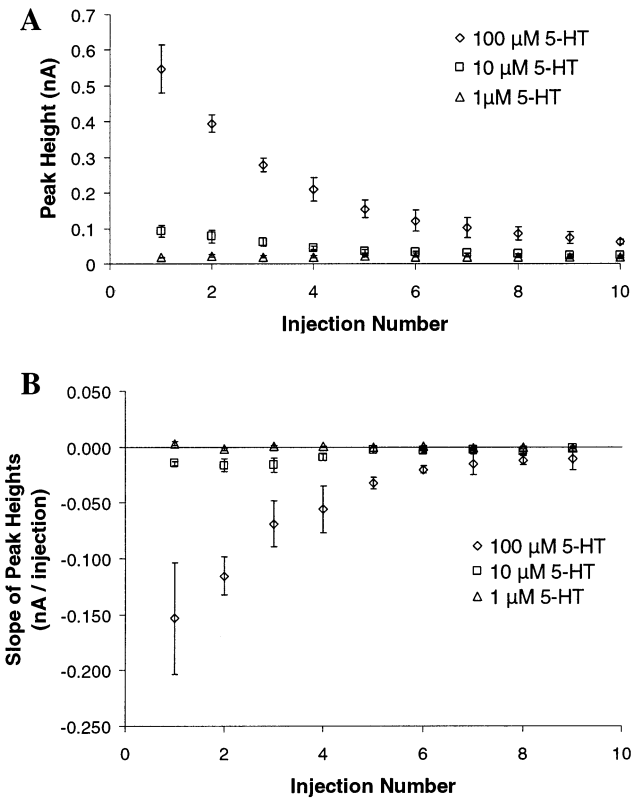

Figure 5. (A) Concentration dependence of electrode fouling: plot of serotonin peak height vs injection number for several concentrations, 100 (◇), 10 (□), and 1 μM (△). (B) Plot of the slope between each injection vs injection number for 10 injections at the concentrations listed in (A). Each point represents the average of three slopes at a specific injection number. Error bars represent the standard deviation of those slopes. Negative slopes indicate greater fouling.

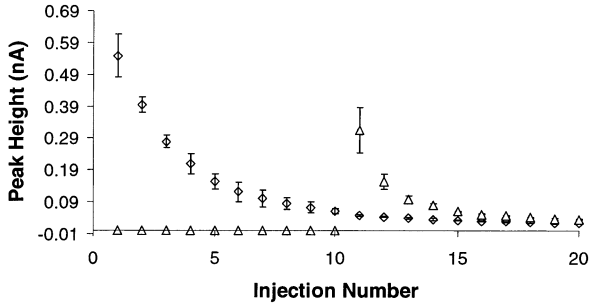

Figure 6. Longevity of the cleaned electrode surface during blank conditions. Beginning with a cleaned electrode surface, 100 μM 5-HT was introduced for injections 1−20 (◇) and 25 mM MES was introduced for injections 1−10, followed by introduction of 100 μM 5-HT for injections 11−20 (△). The response following 10 blank injections is 42.4 $\pm$ 9.8% ($\pm$SD, $n = 3$) that of the control.

Longevity of the Cleaned Electrode Surface. To apply this stripping paradigm to applications such as cellular analysis (in which baseline levels must be established before stimulation and analyte detection), it is important to understand the longevity of the "clean" surface following electrochemical treatment without any analyte present. To test the longevity of the prepared surface, the electrode was treated as before (30 Hz, 30 s), and then 10 blank injections (MES buffer only) were made, followed by 10 injections of 100 μM 5-HT without stripping between any injections. These results are compared to 20 serial injections of 100 μM 5-HT in Figure 6. The first injection of 5-HT following 10 blank injections (~7 min elapsed) is 42.4 $\pm$ 9.8% ($\pm$SD, $n = 3$) the height of the first injection of 5-HT following electrochemical treatment. The loss of sensitivity following blank injections might be attributed to adsorption of gases or contaminants such as trace metals in the buffer or oxidation of the Pt surface.[16] It certainly appears that part of the loss in sensitivity at high concentrations results from simple surface contaminants and part is from analyte oxidation products adsorbing to the electrode. A comparison of Figures 4−6 suggests that the surface passivation attributed only to buffer contaminants and oxides (observed at high serotonin concentrations) does not adversely affect the detection of serotonin at very low concentrations. A possible explanation might involve adsorption sites on the electrode surface with different levels of activity, but our data do not provide any insights here.

Characterization of Stripping Parameters. While the stripping parameters reported thus far have been effective for characterization of fouling, the amount of stripping time (30 s) is impractical for many applications, especially when dynamic events that require stripping between injections are monitored. Figure 7 displays the peak height ($\pm$SD, $n = 3$) versus stripping time (0, 1, 5, 10, 20, and 30 s) for 100 μM 5-HT. The overall trend illustrates that, although increased stripping time appears to increase the response to 5-HT slightly, the greatest improvement in both response and precision is observed with the shortest stripping time. Clearly, the sensitivity rapidly improves dramatically from no stripping to 1 s of stripping, and improvements are more gradual and less reproducible at longer stripping times. Thus, it should be possible to implement a stripping paradigm with little effect on the total analysis time.

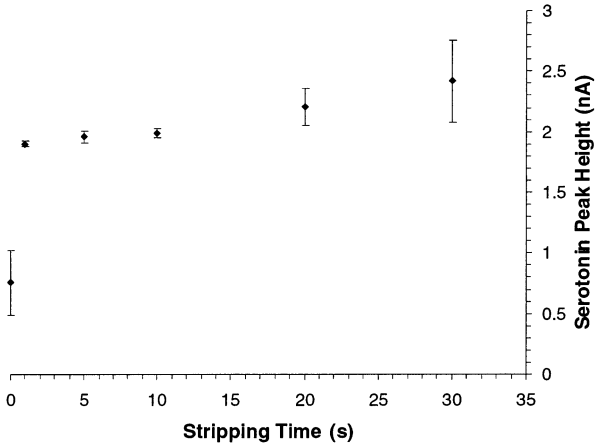

Figure 7. Peak height (±SD, $n = 3$) vs cleaning time (0, 1, 5, 10, 20, 30 s) at constant frequency (30 Hz) for 100 μM 5-HT. The points at 0 s were obtained without electrochemical cleaning following the 1-s cleanings. Due to continuous fouling, each response at 0 s decreases with each consecutive injection as in Figure 1.

Different stripping frequencies have also been investigated to determine the optimal parameters for cleaning the electrode. The electrode was stripped for 5 s at frequencies of 1, 15, 30, and 60 Hz for 100 μM 5-HT. No change in peak height was observed at the different stripping frequencies (data not shown). Therefore, it appears that the electrode response is independent of stripping frequency in the range tested (1−60 Hz).

Limits of Detection Using Electrochemical Treatment. The ability of the cleaned electrode to sensitively and reproducibly measure highly adsorptive molecules has been tested by exploring the limits of detection for 5-HT and Him. It is expected that the calibration curve of highly adsorptive molecules exhibits a negative deviation from linearity at high concentrations. This may be explained by the fact that the analyte is fouling the electrode even as the peak is rising, thereby reducing its height at the apex. Therefore, the linear correlation is greatest at very low concentrations. For collection of the calibration data, the electrode was treated at 30 Hz for 5 s between each injection. The calculated LOD for 5-HT is 78 nM (S/N = 2), with a linear range of 0.1−10 μM ($R^2 = 0.9998$). To our knowledge, this represents the lowest limit of detection of a neurotransmitter to date using microchip CEEC. As described previously, Him appears to adsorb more strongly to the electrode than 5-HT. With regular electrode treatment, however, the LOD for Him is 17 μM (S/N = 2) with a linear range of 25−100 μM ($R^2 = 0.9931$). Therefore, the electrochemical treatment paradigm presented allows relatively sensitive detection of Him at an unmodified Pt electrode, which is extremely problematic without treatment. Amounts of serotonin and histamine released from single rat mast cells have been reported to be 1.6 and 63 fmol, respectively.[42,43] Complete injection of the released material into the separation channel corresponds to approximately 2.90 μM 5-HT and 110 μM Him, well above the limits of detection after electrode pretreatment.

No change in the noise level or adhesion of the electrode to the chip has been observed over the course of using and stripping the electrode (several hundred injections on each chip). The electrodes and chips remained intact and highly responsive well after more than two months of use. The noise level (1.8 pA peak to peak) obtained in this study is the lowest reported noise for microchip CEEC without decoupling.[44] Therefore, it can be concluded that electrochemical treatment as described herein does not adversely affect the performance or longevity of the electrode itself. It has been well established that, over extended use, impurities may become incorporated into the Pt surface in a way that they become nearly impossible to remove.[16] Hence, overall sensitivity was observed to decrease over the lifetime of each chip; although, measurements taken within the same day are comparable as demonstrated herein.

CONCLUSIONS

A method for removing electrogenerated products from Pt electrodes has been demonstrated for electrochemical detection on a microchip. The characteristics of electrode fouling, including response versus injection number and response versus analyte concentration, have been investigated. The results indicate that the performance of the Pt electrode can be reproducibly regenerated following as much as a ∼99% reduction in surface activity. Additionally, it has been demonstrated that electrode fouling may be minimized at very low concentrations (∼1 μM for 5-HT under the conditions tested). Optimization of the electrochemical treatment (a paradigm similar to PAD) has revealed that the greatest benefit and greatest precision are achieved at short cleaning times (1−10 s), while the response appears to be independent of cleaning frequencies from 1 to 60 Hz. The optimized cleaning procedure was applied for the sensitive detection of two compounds that typically present adsorption challenges—serotonin and histamine, the former being detected at nanomolar levels.

Although the electrochemical cleaning procedure was demonstrated for Pt electrodes, the same benefits might be expected for gold electrodes, given their similar response to analytes when performing PAD.[18,19] Repeated use of the electrode and application of electrochemical treatment did not appear to measurably affect the noise, metal adhesion, or physical appearance of the electrode. Additionally, pretreatment of the electrode did not lengthen the time required for the baseline to stabilize following the initiation of the run voltage. It is expected that automation by computer-controlled cleaning, injecting, and running conditions will allow much more rapid separations, making it possible to think about monitoring dynamic cellular release from mast cells.

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